

## Review Article

# *Zataria multiflora*, broiler health and performance: a review

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## Summary

*Zataria multiflora* Boiss. (ZM) from Lamiaceae family, is an herb native to Iran, Pakistan, and Afghanistan with a history of use both as a condiment and a traditional medicine. The herb and/or its preparations have also shown promising effects in modern pharmacological studies. With regard to the importance of reducing synthetic drug administration both as growth promoters and therapeutic agents in poultry industry, this review is a short account of current knowledge about the beneficial effects of this herb on broiler health and performance parameters based on published materials in different databases such as Google Scholar, PubMed, ScienceDirect, Scientific Information Database, Scopus, etc. It seems that ZM, possibly due to the presence of thymol and carvacrol as its main constituents, can affect broiler's health especially with regard to its antioxidant effects, immunomodulatory properties and proper effects on gut bacterial pathogens and/or microbiota. It should be kept in mind that although major constituents of ZM essential oil (as the most studied preparation of the herb) are relatively similar, their relative content may show a broad range that can subsequently affect the outcome of ZM essential oil administration to broilers. Overall, ZM is a pluripotent herb and its effect on broilers should be more clarified in future studies.

**Key words:** Broiler, Health, Immune system, Performance, *Zataria multiflora*

## Introduction

Broiler industry is a fast growing and highly productive process with a major role in providing high quality food for the continuously growing population of the world.

Development of efficient management systems and use of genetically improved chickens with better production traits have substantially improved birds' performance and made it possible for broiler farmers around the world to produce millions of tonnes of chicken meat a year. Despite massive progress, poultry production businesses face many challenges including infectious diseases as one of the most commonly encountered threats that can adversely affect the profitability of the industry due to bird loss, diminished performance of birds as well as the high cost of antimicrobial use specially to treat bacterial diseases.

Mass administration of commercially available antibacterial drugs to broilers in intensive rearing systems is a costly practice and has the potential for inducing a drastic change in gut microbial flora of the bird. On the other hand, health problems for consumers related to antimicrobial residue violations and fast rising antimicrobial resistance are currently two main global concerns. These necessitate the use of alternative strategies to fight or prevent infections (Petrolli *et al.*, 2012).

Herbs, especially those with an ethnopharmacological history or ethnoveterinary use, are usually well tolerated, readily available with low cost, are not a main concern for drug residue and even may improve the appetite of chicken and the quality of the chicken carcass. Moreover, some of the herbs have shown multidimensional positive effects including disease prevention, for instance, by stimulating immune system as well as therapeutic effects. These properties have made herbs "good candidates" for use in broiler industry.

*Zataria multiflora* Boiss. (ZM) colloquially known as "Avishane-e-Shirazi" (Shirazi-thyme or the thyme that belongs to Shiraz, a city in South-West of Iran) in Persian, is an herb with culinary uses and also a long history of use in folk medicine owing to its antimicrobial, analgesic, carminative, anthelmintic and antidiarrheal properties (Sajed *et al.*, 2013). Recently different researchers have become interested in its diverse pharmacological properties and many studies are available with this herb as their main subject. The present review is focused on the current knowledge about the effects of this herb and/or its major constituents (thymol and carvacrol, although these molecules are also present in other herbs such as *Thymus vulgaris*) on health and performance of broilers. Integrating this information can set the scene to find the answer for the question of whether this herb or its constituents can join the league of substances that deserve to be considered as candidates for use in broiler industry?

## Botanical features and phytochemical characteristics

*Zataria multiflora* is a thyme-like herb which belongs to the family Lamiaceae and grows wild in Iran, Afghanistan and Pakistan. The plant is an aromatic shrub with orbicular, densely gland-dotted, ovate leaves and the dense white, hairy, round buds on the leaf axils. Aerial parts of the plant, especially leaves in dried form (Fig. 1), are used as a popular condiment owing to their fascinating aroma and interesting taste.



**Fig. 1:** Dried aerial parts of *Zataria multiflora* with leaves, stems and buds which could be used as a condiment after fine grinding

The plant is especially rich in monoterpenes with usually thymol and carvacrol (a monoterpenoid phenol) as the most abundant constituents (Shokri and Sharifzadeh, 2017). Like other herbs factors including extraction process, time of collection and agricultural characteristics as well as genetic diversity, etc., can affect the composition of the herbal preparation. For instance, Aghamohammadi *et al.* (2016) showed that thymol content of hydro alcoholic extract of ZM is dependent on ethanol concentration in extraction solvent with 70% ethanol as the highest yielding solvent. In another study, Hadian *et al.* (2011) evaluated the essential oil composition of 18 different accessions of ZM collected from different locations in Iran. Based on the composition, the authors divided all accessions into four groups. The first group was characterized by a high content of carvacrol, thymol, and linalool, the second group was dominated by carvacrol, thymol, and p-cymene, the third group was characterized by a high concentration of thymol and a low content of carvacrol and p-cymene, and the fourth group contained linalool and carvacrol as the main components. These authors found 56 different constituents with a relatively wide range of contents. The major constituents were thymol (6.0-54.9%), carvacrol (0.7-50.6%), linalool (1.2-46.8%), and p-cymene (1.6-14.8%). In a closely related study by Kavooosi and Rabiei (2015), on six different chemotypes

of ZM, the main components of ZM essential oil were carvacrol (4-57%), thymol (1-45%), p-cymene (4-20%),  $\gamma$ -terpinene (1-38%), and linalool (1-33%). Sae-Dehkordi *et al.* (2010) reported chemical composition of essential oil from ZM plants collected from different parts of Iran. The essential oil yield ranged from  $0.99 \pm 0.29\%$  to  $1.59 \pm 0.86\%$  v/w. Essential oils from different origins were almost qualitatively similar although some components varied. From different samples, 29 to 53 various compounds were identified that formed 96.5% to 99.5% of total oils in different ecotypes. Thymol was the most abundant component in this study followed by carvacrol and p-cymene.

Alkanes, fatty acids, phytosterols, triterpenes, hydroxycinnamic acids and flavonoids are other plant phytochemicals accompanied by small amounts of tannins, resins and saponins while lacking alkaloids (Sajed *et al.*, 2013).

Essential oil of the plant is the most commonly investigated preparation for its pharmacological effects.

## Effects on immune system of broilers

Immunostimulants can improve the efficiency of poultry industry by preventing the diseases or potentiating the ability of bird to fight back the pathogens. This leads to a lower tendency for use of antimicrobial agents that in turn can decrease the production cost and the chance for drug residue violations and/or antibacterial resistance.

Mosleh *et al.* (2013) evaluated the effect of subcutaneous injections of ZM essential oil at 0.1, 0.2 and 0.4 g/kg/day dosages on antibody titer in broilers vaccinated with B1 vaccine followed by La Sota as well as on delayed type hypersensitivity (DTH) test as a measure of cell-mediated immune response. The authors reported that ZM essential oil stimulates humoral immune responses while suppressing cell-mediated immune responses in chickens vaccinated with live Newcastle disease (ND) vaccines. In a closely related study, Staji *et al.* (2016) investigated the effect of adding different concentrations (100, 200, and ppm) of ZM essential oil to the feed of broilers on antibody titers for sheep red blood cell (SRBC) and ND virus (B1 vaccine) plus stimulation rate of T-lymphocytes by DTH assay. The authors reported that 200 ppm is the optimal dose while 400 ppm showed negative effects, especially with regard to DTH test. Therefore, they suggested the use of low concentrations of ZM for positive immunomodulatory effects. These two studies are coherent and both show that although high doses of ZM can adversely affect cell-mediated immune response, relatively lower doses are associated with promising effects. The reason behind the suppression observed in DTH test may lie in the fact that ZM essential oil is rich in thymol and carvacrol and these monoterpenes can decrease T helper cell-related cytokines/specific transcription factors and subsequently suppress DTH response in mice (Gholijani and Amirghofran, 2016). In chickens, Du *et al.* (2016) evaluated the effect of adding a commercial preparation

containing 25% thymol and 25% carvacrol as active components to the diet at 0, 60, 120, or 240 mg/kg. Birds remained healthy or were orally challenged with *Clostridium perfringens*. The serum antibody titers against ND vaccine, secretory IgA (sIgA) concentrations in intestinal mucosa, intestinal mRNA expression of toll-like receptor (TLR) 2, TLR4, IL-1 $\beta$  and TNF- $\alpha$ , as well as lymphocytes proliferative responses were assayed on day 21. In the challenged birds, supplementation of 120 and 240 mg/kg of the preparation increased the serum antibody titers against ND vaccine. Regardless of challenge, the preparation also linearly inhibited the mRNA expression of TLR2 and TNF- $\alpha$  in the ileum.

Khaksar *et al.* (2012) reported positive effects of adding ZM essential oil to broiler feed (0.1%) on cutaneous basophil hypersensitivity test, heterophil to lymphocyte ratio, white blood cell count and antibody production against SRBC antigen. It is worth mentioning that basophil hypersensitivity test is a distinct form of hypersensitivity reaction with a delayed-time course that is different from both the classic DTH and immediate hypersensitivity. In fact, cutaneous basophil hypersensitivity is mediated by both T and B lymphocytes (Mahapatro and Mahapatro, 1984), while DTH responses in the skin are used to assess cell-mediated immunity *in vivo* which is a T cell-mediated defense mechanism (Ahmed and Blose, 1983).

The immunomodulatory effect of adding ZM to broiler feed was also reported by Ghazvinian *et al.* (2017) as a positive effect on antibody production against SRBC antigen due to administration of ZM at 100 mg/kg of diet for 42 days.

In contrast to enhancing effect of ZM essential oil on antibody response against ND virus or SRBC antigens, administration of ZM essential oil at 20 or 40  $\mu$ L/kg body weight (BW)/day to broilers challenged with avian influenza virus (H9N2 subtype) pre or post viral inoculation, has shown no significant effect on antibody titers in hemagglutination inhibition (HI) test (Shayeganmehr *et al.*, 2018).

The reason behind this discrepancy may be the fact that in H9N2 virus-infected chickens, major histocompatibility complex (MHC) antigens II are down regulated as well as IL-4 receptor and CD74 (MHC class II invariable chain). These molecules are pivotal in the activation of CD4<sup>+</sup> helper T cells and humoral immunity. Remarkably, in H9N2 virus-infected chickens, the antibody response is severely suppressed (Xing *et al.*, 2008).

Aqueous extract of ZM has also been evaluated for its immunomodulatory effects in broilers. In a study by Farzanfar *et al.* (2015), addition of 2 ml/L ZM aqueous extract to drinking water of broilers, increased total anti SRBC and IgM titers on day 21; 1 and 1.5 ml/L increased IgM titer on day 28; 0.5, 1.5 and 2 ml/L increased total anti SRBC and IgG titers on day 35 and 1.5 and 2 ml/L increased IgG titer on day 42. Cell immunity in response to phytohemagglutinin-P (PHA-P) injection was not affected. The authors concluded that ZM aqueous extract has no appreciable effect on cell

immunity but improves humoral immunity in broilers (Farzanfar *et al.*, 2015).

Apart from the studies that have been focused on the effect of ZM on functional aspects of the immune system, some reports are available that show the beneficial effect of this herb on structural features of immune organs in broilers. Supplementation of broiler diet with 2% ZM herb as dried powder during the rearing period has resulted in better histological features of bursa of Fabricius especially the thickness of follicular cortex (Shomali *et al.*, 2013). Similar effects have been reported for caecal tonsils where administration of ZM dried powder during rearing period resulted in shorter and more flattened villi, decreased nodular unit width with increased height, wider follicles in groups that received 1.5 and 2% ZM powder, marked increase in follicle number per nodular unit especially at the 2% level and decreased muscular layer width again mostly in the 2% group (Shomali *et al.*, 2014). Bursa of Fabricius has a pivotal role in B cell development. Cecal tonsils are also important in humoral immune responses. Although at hatch time the predominant cell type in the caecal tonsil is CD4<sup>+</sup> T cells, they are replaced by mainly B cells and a few CD8<sup>+</sup> T cells during development into a mature secondary lymphoid tissue (Jeurissen *et al.*, 1989). Therefore, positive structural changes in these organs by ZM may enhance humoral immunity of broilers.

Taken together, it seems that ZM can potentiate humoral immune responses especially against Newcastle disease virus (NDV), however, high doses of the herb and/or thymol or carvacrol can suppress T-cell mediated immune responses especially by mitigating cytokine levels.

## Birds' performance

As shown in Table 1, different studies have evaluated the effect of administering ZM as dried powder, essential oil or aqueous extract on performance parameters of broiler chickens.

Although the results seem quite contradictory, bewildering and inconclusive at first glance, some points like the type of preparation used (which affects the type and content of the constituents) and especially the dose should be taken into account in order to find the logical trend. As could be deduced from Table 1, when ZM powder was administered to broilers (Ghalyanchi Langeroudi *et al.*, 2008; Salehi *et al.*, 2016), higher doses (1 and 1.5% of the diet) showed negative results, while administering the herb at 0.25% of the diet was associated with positive effect on performance parameters.

The results of using essential oil seem more complex. Whereas Hamdieh *et al.* (2013), have found no significant effect following administration of ZM essential oil at 0.02 and 0.04% of diet, Ghazvinian *et al.* (2017), reported negative results with 0.04% where 0.02% essential oil in diet has resulted in positive effects.

**Table 1:** Effect of *Zataria multiflora* and its preparations on performance parameters of broiler chickens

Preparation	Dose	Performance parameters	Duration of administration	Finding(s)	Reference
Powder	1.5% of diet	BW, feed intake, FCR, mortality, efficiency productive index	42 days	Negative effects	Ghalyanchi Langeroudi <i>et al.</i> (2008)
	0.25, 0.5 and 1% of the diet	BW gain, feed intake, FCR	42 days	Negative effects on feed intake and weight gain by 1%, improved results due to 0.25%	Salehi <i>et al.</i> (2016)
Essential oil	0.02 or 0.04% of diet	BW, FCR, livability, and production index	42 days	No significant effect	Hamdieh <i>et al.</i> (2013)
	0.01, 0.02 and 0.04% of diet	BW gain, feed intake, FCR, final weight	42 days	Positive results with 0.02% of diet on weight gain (whole period) and FCR (only in starter (0-14 days of age) period), while administration of 0.04% was associated with negative effects especially on BW	Ghazvinian <i>et al.</i> (2017)
	20 or 40 µg/kg/day, orally	BW, feed intake, FCR	From day 21 or 27 in birds challenged with avian influenza virus at day 25 until day 40 of age	Positive effects	Shayeganmehr <i>et al.</i> (2018)
Aqueous extract	0.5 and 1% of diet	BW, feed intake, FCR	42 days, last 14 days of rearing, last 7 days of rearing	1% for the 42 days improved FCR, both doses for 42 days reduced feed intake, no significant effect on BW	Mohammadpour <i>et al.</i> (2015)
	0.5, 1, 1.5 and 2 ml/L of drinking water	Feed intake, weight gain, FCR	42 days	No significant effect	Farzanfar <i>et al.</i> (2015)
	0.05% of diet	Feed intake, weight gain, FCR	42 days and final 2 weeks of rearing	No significant effect	Nobakht <i>et al.</i> (2017)

BW: Body weight, and FCR: Feed conversion ratio

This difference may be related to the composition of the basal diet and environmental conditions and above all the different levels of each constituent of the essential oil used. Although the analysis of the essential oil was not performed in the study by Ghazvinian *et al.* (2017) the former report by Hamdieh *et al.* (2013) included the GC/Mass analysis of the essential oil and showed that the thymol and carvacrol contents of the preparation were 22.3% and 3.1%, respectively. Lee *et al.* (2003), evaluated the effect of diets supplemented with 200 ppm thymol or carvacrol on female broilers performance. In this study dietary carvacrol lowered feed intake and weight gain as well as the feed-to-gain ratio. Dietary thymol, did not affect growth performance. Therefore, with regard to very low carvacrol content of the essential oil that was used in the study by Hamdieh *et al.* (2011) the insignificant change in performance parameters seems quite anticipated.

Effects of thymol and carvacrol on broilers' performance have also been evaluated. In 2013, Hashemipour *et al.*, evaluated the effect of administering an equal mixture of thymol and carvacrol at 4 levels (0,

0.006%, 0.01%, and 0.02% of diet) from day 0 to 42 on performance of broiler chickens (Hashemipour, 2013a). The inclusion of thymol + carvacrol linearly decreased feed intake, but the highest BW gain and feed efficiency was observed in broilers offered 0.02% of product. In another study by Hashemipour *et al.* (2016) effect of broiler feed supplementation with the same mixture at 0.01% and 0.02% of diet with or without a non-starch polysaccharides (NSP)-degrading enzyme product was investigated from day 0 to 42. Compared with the control group, birds that were fed with diets containing thymol and carvacrol had a higher final BW, average daily gain and feed efficiency. Dietary supplementation with thymol and carvacrol increased total volatile fatty acids and acetate levels at day 24 and 42, whereas the level of butyrate decreased. The authors suggested that thymol and carvacrol, with or without an NSP-degrading enzyme, improve growth performance in broilers fed on a wheat-based diet. Taken together, it seems that apart from the dose, the ratio of thymol to carvacrol may be important in determining the effects of the preparation on broilers performance.

Except for the effect of administering 1% ZM aqueous extract in diet on feed conversion ratio (FCR), results of other doses or routes of this preparation have been disappointing in broilers.

## Gastrointestinal histology, microbiota and bacterial diseases

Gut histology and microbial status can affect broiler performance by different mechanisms such as determining the capacity of nutrient absorption for the former, aiding in digestion, vitamin synthesis, immune training and organ development for the latter (Owens *et al.*, 2008; Hamed *et al.*, 2011).

The effect of administering ZM in broilers' diet on histomorphometric parameters of intestine has been clearly described by Hamed (2013). In this study, diets containing 0.5, 1, 1.5, and 2% of ZM dried powder (experimental groups) or basal diet were used for 40 days to feed broilers. Thymol and carvacrol contents of the herb were not quantified. Height and width of villi, depth of crypts, and width of sub-mucosal and muscular layers were measured in duodenum, jejunum and ileum. Although few changes were observed due to ZM administration, the villus height and villus height/crypt depth ratio showed no significant change in three different parts of the intestines. Villus height can affect bird performance in a way that longer villi have an increased digestive and absorptive function due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems (Amat *et al.*, 1996). Therefore, it seems that ZM at least at these dosages cannot influence intestinal micro structures which are involved in nutrient absorption. Khaksar *et al.* (2013), evaluated the effect of adding ZM essential oil at 0.1% to broilers diet on histological features of ileum and jejunum after 21 and 42 days. These authors detected changes in villus height and villus height/crypt depth ratio especially in ileum. Although ileum has absorptive capacity, jejunum has the highest enzyme content for digestion and the highest capacity for fatty acid, calcium, phosphate and some other mineral absorption with duodenum as the most effective part of intestine for glucose absorption (Scanes, 2015), therefore, it does not seem that ZM essential oil may have a remarkable effect on birds' ability for nutrient absorption. Another point that deserves to be mentioned here is that the content of thymol and carvacrol that was used in this study were about 22% and 32%, respectively (a ratio of ~ 0.7:1). In a study by Hashemipour *et al.* (2013b), inclusion of 100 and 200 mg/kg of a preparation containing equal amounts of thymol and carvacrol (1:1), as the active ingredients, improved villus height, villus surface, villus height/crypt depth ratio and muscular layer of jejunum at 21 and 42 days of age with almost similar effects on ileum. In a study by Du *et al.* (2016); administration of a preparation with 25% thymol and 25% carvacrol at 60, 120, or 240 mg/kg of diet to broilers challenged with *C. perfringens*, linearly alleviated the gut lesions and improved the ratio of villus height to crypt depth in

ileum. However, this preparation did not affect villus height to crypt depth ratio in normal broilers.

Taken together, these controversies may imply that besides the dosage, the ratio of thymol to carvacrol may be important in the effects of ZM or its preparations on gut histology.

Studies on the effect of ZM on gastrointestinal microbiota have shown more promising results.

Mohammadpour *et al.* (2015), observed that administration of aqueous extract of ZM at 0.5 and 1% of broiler diet can reduce *Escherichia coli* while increasing *Lactobacilli* populations of ileum after both 21 and 42 days. Same results were observed after addition of ZM aqueous extract in normal or high fat broiler diets at 0.5% of diet for 42 days (Nobakht *et al.*, 2016).

Yin *et al.* (2017); showed that supplemental thymol and carvacrol (25% thymol and 25% carvacrol) at 120 mg/kg of diet can change the ileum *Lactobacilli* population by increasing the numbers of *L. crispatus* and *L. agilis*, and decreasing *L. salivarius* and *L. johnsonii*. Moreover, this preparation could reduce the effect of necrotic enteritis caused by *C. perfringens* in chickens.

In a study by Hashemipour *et al.* (2016) supplementing broiler diet with 200 mg/kg of thymol and carvacrol resulted in lower *E. coli* and *C. perfringens* counts accompanied by higher *Lactobacilli* counts in broilers' cecal samples.

In a broiler infection model with *C. perfringens*, the diets supplemented with encapsulated carvacrol at the dose of either 250 or 650 µg/g significantly decreased necrotic enteritis lesions in chickens, which was close to the bacitracin/salinomycin treated birds, without significant impact on intestinal burden of *Lactobacillus*. However, the low dose of encapsulated carvacrol decreased *C. perfringens* count in the ileum of birds at 35 days of age (Liu *et al.*, 2016).

In a closely related study, dietary supplementation of broilers challenged with *C. perfringens* with a product containing 25% thymol and 25% carvacrol as active components at 0, 60, 120, or 240 mg/kg of wheat-based diet, did not influence the *C. perfringens* bacterial count, but linearly alleviated intestinal lesions on day 21 and 28, and decreased *Escherichia* populations in ileum with increased dosages. For caecum, the preparation quadratically influenced *Lactobacillus* populations on day 21, and linearly decreased total bacterial count and *Escherichia* on day 28 (Du *et al.*, 2015).

Arsi *et al.* (2014) evaluated the efficacy of thymol and carvacrol against *Campylobacter* colonization in broiler chickens. On days 3 and 10 of age, birds were orally challenged with *C. jejuni*; cecal samples were collected for bacterial count. *Campylobacter* counts were reduced for 0.25% thymol, 1% carvacrol or 2% thymol treatments, or a combination of both thymol and carvacrol at 0.5%.

It has been shown that carvacrol-rich ZM essential oils exhibit better *in vitro* antibacterial activities (Kavoosi and Rabiei, 2015).

## Antiviral effects

Although it cannot be considered as a direct antiviral effect, in 2013, Mosleh *et al.*, showed that administration of ZM essential oil can shorten faecal virus shedding in broilers vaccinated with live ND vaccines (B1 vaccine followed by La Sota 10 days later). The effect of ZM essential oil has also been evaluated against NDV on Vero cells in a time of addition-experiment (Mohammadi *et al.*, 2015). In this study, ZM essential oil at concentrations of 1/5000, 1/25000, 1/125000 or 1/625000 was added at different times of infection: 60 min pre infection, simultaneously and 60 min post infection. The most prominent finding of the study was that median tissue culture infective dose (TCID<sub>50</sub>) values of all treatments were very close to that of control virus except for simultaneous administration at concentration of 1/5000 which reduced TCID<sub>50</sub> about 1000 fold. The essential oil had a roughly similar amount of thymol and carvacrol (about 30%).

In a study by Shayeganmehr *et al.* (2018), broilers received either 20 or 40 µL/kg BW/day ZM essential oil seven days before the challenge with two other groups received the essential oil at the same dosage two days after H9N2 challenge (on day 25 of age). Authors reported reduced viral replication in the respiratory and gastrointestinal tracts compared to the control. Thymol and carvacrol contents of the essential oil which was used in this study were 47% and 22%, respectively.

It has been previously shown that thymol, carvacrol, p-cymene have antiviral effects for instance against herpes simplex virus type 1 (HSV-1), with thymol showing the highest potency as demonstrated by a half maximal inhibitory concentration (IC<sub>50</sub>) of 0.002% (Sharifi-Rad *et al.*, 2017).

Regarding the fact that these molecules are major constituents of ZM essential oil, we may hypothesize that they could be important in antiviral effects against avian viruses. Apart from the virus type and the status of the immune system of the host, since the percent of these substances could be different in essential oil preparations, difference in the magnitude of antiviral effects seems quite expected. Collectively, elucidation of possible effects of ZM or its preparations against avian viruses needs further studies.

## Antioxidant properties

Along with the studies that have approved the antioxidant properties of ZM both *in vivo* and *in vitro*, some studies have shown antioxidant effects of ZM in broilers.

In a study by Nobakht *et al.* (2017b) administration of ZM aqueous extract at 0.5% of diet for 42 days resulted in reduced malondialdehyde (MDA) concentration accompanied by an increase in glutathione peroxidase activity in serum of broilers fed with high fat or normal diets. The authors also showed the same results on MDA concentration when ZM extract added only for the last two weeks of rearing period. In another

study, Jebelli Javan *et al.* (2012) showed that feeding broilers with diets containing 100, 200, and 400 mg/kg ZM essential oil for 42 days reduces MDA content of breast meat. In a study by Luna *et al.* (2010), supplementation of broilers' diet with 150 mg/kg of thymol, or 150 mg/kg of carvacrol resulted in lower values of thio-barbituric acid-reactive substances (TBARS) in meat after 5 and 10 days of storage.

In a study by Kavooosi and Rabiei (2015) it was shown that thymol-rich ZM essential oils exhibit better antioxidant activity.

## Miscellaneous effects

In 2013, Hosseini *et al.*, observed that administration of ZM essential oil at 200 and 400 mg/kg of broiler diet for 42 days significantly reduces ascites heart index (AHI, right ventricular weight/total ventricular weight) which is a sensitive indicator of prior exposure of the heart to increased pulmonary arterial pressures (Burton *et al.*, 1968). The mean values of this indicator were 0.179 and 0.191 for 200 and 400 mg/kg ZM groups vs. 0.268 for control group. It should be stated that broilers with an AHI<0.27 without fluid in the abdomen are regarded as normal, whereas those with an AHI≥0.30 with fluid accumulation are regarded as having pulmonary hypertension (Cawthon *et al.*, 2001). No difference in blood packed cell volume (PCV) was observed in this study.

## Conclusion

It seems that ZM and/or its preparations possess the criteria to be considered as a plausible source for biologically active substances with beneficial effects on broiler industry as well as the public health with reducing the need for drug use and subsequent hazard of drug residue violations, antimicrobial resistance, etc. Taken together, this herb shows promising results for applications in both organic and conventional poultry productions. It should be kept in mind that although major constituents of ZM essential oil are relatively similar, their content shows a broad range in different preparations that can affect the magnitude or even the type of the effect illustrated by ZM essential oil. This can at least partially describe some of the inconsistencies observed when this herb is used in broilers. Overall, ZM is a pluripotent herb that many of its positive and/or adverse effects, their mechanisms as well as the exact active constituent(s) for each effect, remain to be clarified in future studies.

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